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Research Paper

Polyisoprenoid Compounds from Tropical Fruit Trees in Universitas Sumatera Utara Campus

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Abstract
Tropical trees are a source of secondary metabolite compounds that have various biological activities that can help human life both for industrial and pharmaceutical needs. One of the secondary metabolites is polyisoprenoid. This study aims to identify and analyze polyisoprenoid compounds quantitatively from tropical fruit trees at the Universitas Sumatera Utara. Nine samples of tropical fruit were obtained, namely: *Psidium guajava, Tamarindus indica, Manilkara kauki, Morinda citrifolia, Mangifera indica, Artocarpus communis, Artocarpus heterophyllus, Gmelina arborea,* and *Syzygium aqueum*. Each sample was extracted and isolated the polyisoprenoid alcohol, then analyzed using two-dimensional thin-layer chromatography. The polyisoprenoid in fruit samples found at the Universitas Sumatera Utara was a type II and III polyisoprenoid compound. The total lipid values ranged from 48.7 mg/g dw to 262.9 mg/g dw, polyisoprenoids ranged from 1.2 mg/g dw to 9.5 mg/g dw, and polyprenols ranged from 0.5 mg/g dw to 5.7 mg/g dw. Carbon chain length and dolichol polyprenol lengths of each fruit were collected (*Psidium guajava L.*) ($C_{70}-C_{80}$), (*Tamarindus indica L.*) ($C_{80}-C_{95}$), (*Manilkara kauki L.*) ($C_{80}-C_{95}$ and $C_{70}-C_{90}$), (*Morinda citrifolia L.*) ($C_{75}-C_{100}$), (*Mangifera indica L.*) ($C_{80}-C_{90}$ and $C_{75}-C_{90}$), (*Artocarpus communis*) ($C_{75}-C_{95}$), (*Artocarpus heterophyllus*) ($C_{70}-C_{75}$ and $C_{70}-C_{100}$), (*Gmelina arborea Roxb.*) ($C_{90}-C_{95}$), and (*Syzygium aqueum*) ($C_{65}-C_{90}$ and $C_{75}-C_{90}$).

Keywords
Polyisoprenoid, Dolichol, Polyprenol, Tropical Fruits, Two-Dimensional Thin-Layer Chromatography, Carbon Chain Length

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1. INTRODUCTION

Indonesia is a home for the second-largest humid tropical forest in the world which is rich in biodiversity, especially plants (Riswan and Yamada, 2006). Indonesia is known as one of the seven mega biodiversity countries in the world with a higher plant distribution of more than 12% (30,000 species) than those on earth (250,000 species) (Paramita and Rahmadi, 2020). In tropical countries, trees are widely used as traditional medicine by local communities as ingredients derived from their leaves, stems, roots, seeds, and flowers (Widiyati, 2006; Meliki and Lovadi, 2013).

Tropical trees are known to be a source of secondary metabolite compounds (Mulyani et al., 2013). Secondary metabolites are natural compounds that are synthesized by living things such as plants, microbes, or animals to pass through the biosynthetic process which is used to support life (not vital). Terpenoids, polyisoprenoids, polyketides, alkaloids, flavonoids, and steroids are some secondary metabolite compounds (Mulyani et al., 2013; Saifudin, 2012). Secondary metabolites are needed by humans, both for industrial use and medicinal substances. This feature is because these secondary metabolites have various biological activities, including anticancer, antibacterial, antioxidant, and antifungal (Febrina et al., 2015). Due to the biological activity of isoprenoids themselves, such as triterpenoids and phytosterols, it is considered important as a natural potential resource for medicinal compounds (Sparg et al., 2004).

Polyisoprenoid alcohol belong to a secondary metabolite (Swiezewska and Danikiewicz, 2005). Polyisoprenoid plays an important role in plant protection, especially protecting biological membranes that work together with carotenes and tocopherols from lipids and other proteins (Peñuelas and Munné-Bosch, 2005). In plants, synthetic isoprenoid metabolism is involved in the biosynthesis of chlorophyll and various plant hormones, antioxidants, and essential, and helps produce pigments and aromas in flowers and fruit (Jones et al., 2009). So far, research on long-chain polyisoprenoid has focused more

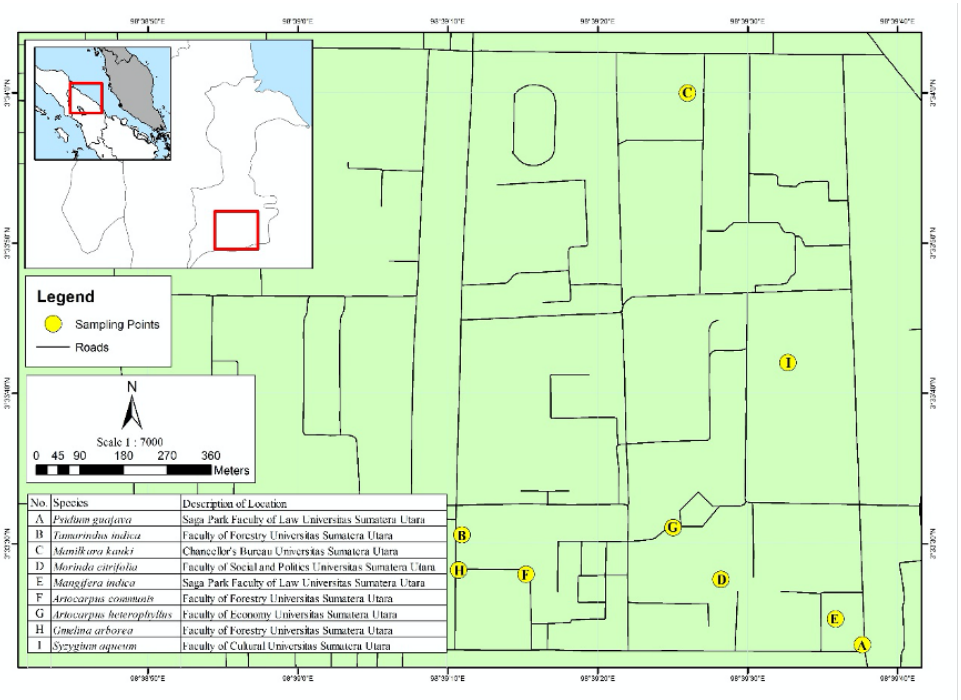

Figure 1. Fruit Samples Were Collected Around The Universitas Sumatera Utara Area. *Psidium guajava* (A), *Tamarindus indica* (B), *Manilkara kauki* (C), *Morinda citrifolia* (D), *Mangifera indica* (E), *Artocarpus communis* (F), *Artocarpus heterophyllus* (G), *Gmelina arborea* (H), and *Syzygium aqueum* (I).

on bacteria, mammals, animals, and cultured cells.

However, there is still little research on any plant tissue, especially tropical and subtropical plants. Therefore, it is necessary to carry out a detailed analysis of tropical fruit trees on polyisoprenoid tropical trees at the Universitas Sumatera Utara to provide more knowledge about the importance of using tropical trees as a source of biologically active compound.

2. EXPERIMENTAL SECTION

2.1 Material

2.1.1 Chemical Material

The standard compound mixture of dolichol (C_{90}−C_{105}) and polyprenol (C_{90}−C_{100}) was used to clarify polyisoprenoids in this work, as earlier reported (Basyuni et al., 2016). Silica gel 60 thin layer chromatography (TLC) plates and RP-18 reverse-phase silica thin layer chromatography (HPTLC) were obtained from Merck (Darmstadt, Germany). The chemicals and other solvents were from the reagent class and were purchased from Merck. Identification of family files according to polyprenol or dolichols was carried out for at least three trials.

2.1.2 Plant Material

Fruit samples were collected around the Universitas Sumatera Utara area from October 2018 - March 2019 (Figure 1 and 2). Fruit samples tested were:12-year-old *Psidium guajava L.* (A), 20-year-old *Tamarindus indica L.* (B), 8-year-old *Manilkara kauki L.* (C), 7-year-old *Morinda citrifolia L.* (D), 15-year-old *Mangifera indica L.* (E), 8-year-old *Artocarpus communis* (F), 10-year-old *Artocarpus heterophyllus* (G), 15-year-old *Gmelina arborea Roxb.* (H), and 7-year-old *Syzygium aqueum* (I). To keep samples fresh, store them in the refrigerator before use. To facilitate purification, the sample was cut into small pieces and oven/dried at 70°C for 72 hours (3 days). The dried sample was then crushed using a grinding machine (Foss).

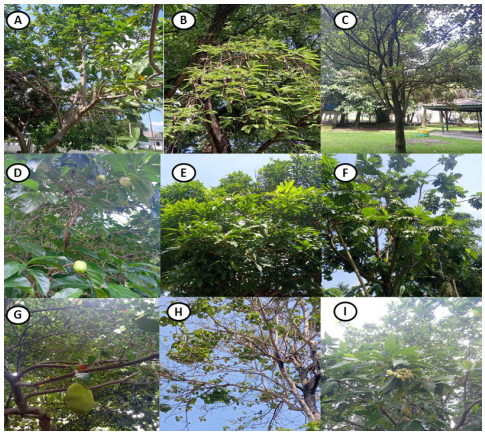

Figure 2. Fruit Samples Tested, *Psidium guajava* (A), *Tamarindus indica* (B), *Manilkara kauki* (C), *Morinda citrifolia* (D), *Mangifera indica* (E), *Artocarpus communis* (F), *Artocarpus heterophyllus* (G), *Gmelina arborea* (H), and *Syzygium aqueum* (I).

Table 1. Total Lipid Value and Distribution of Polyprenol and Dolichol in Tropical Fruits at Universitas Sumatera Utara

Species	TL (mg/g dw)	PI (mg/g dw)	Pol (mg/g)	Dol (mg/g)	% in Total Lipid			% Polyisoprenoid		Group
					Poly	Pol	Dol	Pol	Dol	
P. guajava	56.6 ± 8.0	3.1 ± 0.3	3.1± 0.3	nd	5.5	5.5	nd	100	nd	III
T. indica	61.0 ± 20	4.9 ± 0.6	3.9± 0.7	1.0 ± 0.7	8	6	2	79.8	20.2	II
M. kauki	86.6 ± 66	3.2 ± 1.2	1.2 ± 0.6	2.0 ± 1.0	3.7	1.4	2	37.5	62.5	II
M. citrifolia	262.9 ± 17.8	1.2 ± 0.4	1.2 ± 0.4	nd	0.5	0.5	nd	100	nd	III
M. indica	71.3 ± 40.6	4.3 ± 1.1	2.1 ± 0.7	2.2 ± 0.4	6	2.9	3	48.6	51.4	II
A. communis	48.7 ± 3.9	4.4 ± 0.6	4.4 ± 4.0	nd	9	9	nd	100	nd	III
A. heterophyllus	54.6 ± 7.7	3.7 ± 0.5	1.9 ± 1.6	1.8± 1.5	6.8	3.5	3	53.1	46.9	II
G. arborea	79.3 ± 4.6	1.6 ± 0.6	1.6 ± 0.6	nd	2	2	nd	100	nd	III
S. aqueum	59.5 ± 4.9	9.5 ± 2.9	5.7 ± 1.9	3.8 ± 2.1	16	9.5	6	59.8	40.2	II

*dw = dry weight, nd = not detected, TL = Total Lipid, Poly = Polyisoprenoid, Pol = Polyprenol, and Dol = Dolichol

2.2 Methods

2.2.1 Lipid Extraction

Fruit (5 g of dry weight), each extracted with chloroform: methanol ($CHCl_3$: CH_3OH with a volume ratio of 2:1, CM_{21}) (Floch, 1957). Then incubated at 40°C for 24 hours. Cell walls containing impurities that are insoluble in CM_{21} are filtered with No.2 filtration paper (Advantec, Tokyo, Japan) and what remains is the lipid extract in chloroform, which will then be saponified (Basyuni et al., 2019). Concentrated fat extract liquid was dried at 70°C for 24 hours, then weighed and the lipid weight was determined to determine the total fat/tissue content (mg/g of tissue).

2.2.2 Saponification

The fruit fat extract was saponified at 65°C for 24 hours with the ratio: (2 mL water, 2 mL ethanol, 0.45 g KOH)/sample. After 24 hours of incubation in a water bath, the samples were oven-dried at 50-55°C for completely dry samples.

2.2.3 Analysis by Two-Dimensional Chromatography

The first dimensional TLC was performed for 50 minutes on silica gel (20 x 3 cm) with a toluene-ethyl acetate (9:1) solvent system (Sagami et al., 1992). In the TLC analysis, the polyprenol family moved more quickly than the dolichol family. The longitudinal edge of the first dimensional TLC with a width of 1 cm and the concentration zone of reverse phase TLC C-18 were clamped using two magnetic bars (4.0 x 1.1 x 0.8 cm) facing each gel phase. The bound TLC plate is then expanded perpendicular to the first dimension to transfer the polyprenol and dolichol to the concentration site of the reverse TLC phase.

The second dimension of the reserving RP-18-TLC silica phase with acetone solvent for approximately 30 minutes. Iodine vapor was used to separate and expand the locations of the polyisoprenoid alcohol. The chromatographic images were obtained and scanned digitally with the Epson L200 printer series. The contents of polyprenol and dolichol detected on HPTLC RP-18 were calculated using ImageJ 1.46r with dolichol and polyprenol standards as references (Schneider et al., 2012).

3. RESULTS AND DISCUSSION

3.1 Polyisoprenoid Analysis of Fruit

Separation of polyisoprenoid into polyprenols and dolichols using the 2D-TLC (two-dimensional thin-layer chromatography as previously described (Sagami et al., 1992; Basyuni et al., 2016) to show the profile of polyprenols and dolichols in tropical fruit around the area of Universitas Sumatera Utara. Table 1 displayed the total lipid value and the value of polyisoprenoid, polyprenol, and dolichol from each fruit grown varied among the species.

Polyisoprenoid in fruit samples has type II and type III. Similar to previous research by Basyuni and Wati (2017), *Nephellium lappaceum* roots, fruit skins, and leaf tissue types I, II, and III were detected. The pattern of polyprenol and dolichol is categorized into three groups (I, II, III). Type I shows a dominance of dolichol compounds of more than 90%, type II contains polyprenol and dolichol compounds both in plant tissue, while type III is predominated by polyprenol compounds of more than 90% (Basyuni et al., 2016; Basyuni et al., 2019). Arifiyanto et al. (2017) stated that the difference in the number of polyisoprenoid compounds in the distribution of dolichol and polyprenol should not be the same, the factors that influence these differences are due to age and tissue type or different environmental conditions so that the distribution is also different. Each plant tissue contains different polyprenol and dolichol compounds because differences in the age of each tissue and environmental conditions from one plant to another make differences in polyisoprenoid content. Jankowski et al. (1994) detected the differences carbon chain-length of polyprenols in young leaves and old leaves in leaves of the Potentilla genus. Polyprenols were found in Spermatophyta seeds, while in monocot seeds. By contrast, polyprenols and a small amount of dolichol were detected. This circumstance shows that the difference occurred in tissue age and tissue type. The difference in the number of leaf polyprenols in the dry season and winter is different, this finding suggested that environmental conditions affect the number of polyprenols (Basyuni et al., 2016).

The total lipid values ranged from 54.6 mg/g dw to 262.9

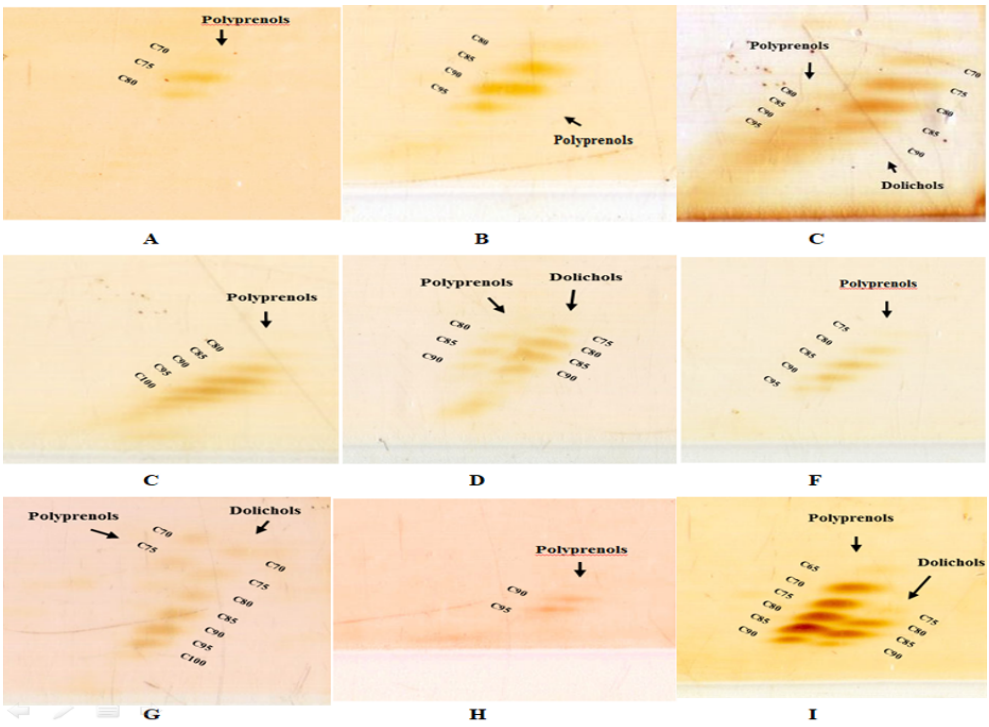

Figure 3. Two-Dimensional Thin Layer Chromatography (2D-TLC) Polyisoprenoid Alcohol Types of *Psidium guajava* (A), *Tamarindus indica* (B), *Manilkara kauki* (C), *Morinda citrifolia* (D), *Mangifera indica* (E), *Artocarpus communis* (F), *Artocarpus heterophyllus* (G), *Gmelina arborea* (H), and *Syzygium aqueum* (I). The Carbon Number Indicates to The Length of The Polyisoprenoid Carbon Chain.

mg/g dw. The highest total lipid was found in *Morinda citrifolia L.* (D), which was 262.9 mg/g dw. The largest total lipid in polyisoprenoid was found in *Syzygium aqueum* (I), namely 15.9%. The largest total polyprenol and dolichol lipids were found in *Syzygium aqueum* (I), namely 5.7 mg/g dw for polyprenol and 3.8 mg/g dw for dolichol. The smallest total polyisoprenoid and polyprenol lipid were *Morinda citrifolia L.* (D), which was 1.2 mg/g dw. The smallest total dolichol lipid was found in the type of *Tamarindus indica L.* (B), which was 1.0 mg/g dw. Lipids in cell membranes can play an important role in the adaptation of plants to environmental stresses (Liu et al., 2019).

The polyisoprenoid in fruit obtained at Universitas Sumatera Utara (Table 1) comprised from 0.48 mg/g dw to 9.49 mg/g dw. Polyprenol in the fruit collections at the Universitas Sumatera Utara composed from 1.2 mg/g dw to 9.5 mg/g dw. Meanwhile, not all of the collected fruit contained dolichol, only a few fruits contained dolichols such as *Tamarindus indica L.* (B), *Manilkara kauki L.* (C), *Mangifera indica L.* (E), *Artocarpus heterophyllus* (G), and *Syzygium aqueum* (I).

3.2 Polyisoprenoid Compounds Using Two-Dimensional-Thin Layer Chromatography Analysis

The polyisoprenoid positions were separated and formed the freckles, from large spots to small spots with acetone solvent on silica gel and visualized in color using iodine vapor. The image was then scanned using a printer scan and the amount of polyprenol and dolichol calculated using ImageJ application with dolichol and polyprenol standards as reference (Figure 3).

Polyprenol compounds were detected more in fruit than dolichol compounds (Basyuni et al., 2016). It has been shown in the plant world (especially leaf tissue), polyprenols are generally found in higher concentrations than dolichol (Basyuni et al., 2016). Polyprenol and dolichol compounds detected in fruit samples had different carbon chain lengths (C) for each sample. According to Tateyama et al. (1999), the occurrance of long-chain polyprenols is not necessarily the same as long-chain dolichols with the same concentration compared to dolichols.

3.3 Carbon Chain Length Analysis

The results of the two-dimensional thin-layer chromatography calculation showed that for samples of fruit types obtained at the Universitas Sumatera Utara, not all fruit samples had dolichol and polyprenol compounds (Figure 3). Several fruit samples of *Psidium guajava L.* (A), *Tamarindus indica L.* (B), *Morinda citrifolia L.* (D), *Artocarpus communis* (F), and *Gmelina arborea Roxb* (H). there are only polyprenol compounds called type III (Table 1). In the analysis of polyisoprenoid on fruit at the Universitas Sumatera Utara, it can be seen that the main components of polyisoprenoid are polyprenol and dolichol compounds. However, the profile of long-chain polyprenols is necessarily the different as long-chain dolichols in the same tissue, the concentration of polyisoprenoids in plants changes

Table 2. Length of Polyprenol and Dolichol Carbon Chain in Tropical Fruits at Universitas Sumatera Utara Campus

Species	Tissue	Polyprenol								Dolichol							
P. guajava L.	Fruit		70	75	80												
T. indica L.	Fruit				80	85	90	95									
M. kauki L.	Fruit				80	85	90	95		70	75	80	85	90			
M. citrifolia L.	Fruit				80	85	90	95	100								
M. indica L.	Fruit				80	85	90				75	80	85	90			
A. communis	Fruit			75	80	85	90	95									
A. heterophyllus	Fruit		70	75						70	75	80	85	90	95	100	
G. arborea Roxb.	Fruit						90	95									
S. aqueum	Fruit	65	70	75	80	85	90				75	80	85	90			

due to differences in aging and season (Basyuni et al., 2016).

The fruit samples of *Manilkara kauki L.* (C), *Mangifera indica L.* (E), *Artocarpus heterophyllus* (G), and *Syzygium aqueum* (I) had both compounds (polyprenol and dolichol) called type II (Table 1). The length of the polyprenol and dolichol carbon chains can be seen in Table 2. For *Psidium guajava L.* (A) samples, the only polyprenol compounds with carbon chain length $C_{70}-C_{80}$. For the *Tamarindus indica L.* (B) sample, there were only polyprenol compounds with a carbon chain length of $C_{80}-C_{95}$. For the *Manilkara kauki L.* (C) sample, there are dolichol and polyprenol compounds with carbon chain lengths in polyprenol compounds $C_{80}-C_{95}$ and carbon chain lengths in dolichol compounds $C_{70}-C_{90}$. *Morinda citrifolia L.* (D) samples only contained polyprenol compounds with carbon chain lengths, namely $C_{75}-C_{100}$. For *Mangifera indica L.* (E) fruit samples, the carbon chain length of the polyprenol compound $C_{80}-C_{90}$ and dolichol $C_{75}-C_{90}$. The sample of Artocarpus communis (F) has a carbon length of $C_{75}-C_{95}$ in polyprenol compounds.

There are two types of *Artocarpus heterophyllus* (G), namely dolichol with carbon chain length $C_{70}-C_{100}$ and polyprenol with carbon chain length $C_{70}-C_{75}$. In the type of *Gmelina arborea Roxb.* (H), there is a polyprenol compound with a carbon chain length of $C_{90}-C_{95}$. For the type of *Syzygium aqueum* (I), there are two compounds, namely polyprenol $C_{65}-C_{90}$ and dolichol with carbon chain length $C_{75}-C_{90}$. Based on the results of carbon chain length calculations (Table 2), polyprenol compounds have carbon chain lengths $C_{65}-C_{100}$ and dolichol compounds have carbon chain lengths $C_{70}-C_{100}$. Polyisoprenoid composition has been independently regulated in the plant kingdom, including in the fruit tested in this study (Floch, 1957; Tateyama et al., 1999; Basyuni and Wati, 2017).

4. CONCLUSIONS

The polyisoprenoid in nine fruit samples found at the Universitas Sumatera Utara were type II and III polyisoprenoid compounds. Types that have both polyprenol and dolichol compounds (type II) are *Manilkara kauki* (C), *Mangifera indica* (E), *Artocarpus heterophyllus* (G), and *Syzygium aqueum* (I). While the types that only have polyprenol compounds (type III), without dolichol are: *Psidium guajava* (A), *Tamarindus indica* (B), *Gmelina arborea* (H), *Artocarpus communis* (F), and *Morinda citrifolia L.* (D).

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